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Indian Standard

# SPECIFICATION FOR RAW SEAWEEDS PART 1 AGAROPHYTES

#### 0. Foreword

0.1 Seaweeds for commercial usage are classified into two types (a) Agarophytes which are the agar-yielding seaweeds, and (b) Alginophytes which are the alginate-yielding seaweeds.

This standard is being dealt with in the following two parts:

Part 1 Agarophytes

Part 2 Alginophytes

- **0.2** This part (Part 1) covers agarophytes. Agarophytes are harvested regularly from patural habitats. Till today, aqua-cultured species have not been used to produce agar in India. Different species have different quality and quantity of agar. Seasonal variations have also been observed.
- **0.2.1** A method for determining the quality index and basis of price fixation has been given in Appendix A for guidance.
- 1. Scope  $\rightarrow$  This standard ( Part 1 ) prescribes the requirements and methods of sampling and test for agarophytes ( raw agar-yielding seaweeds ).
- 2. Types and Grades This part (Part 1) covers agarophytes of *Gracilaria* and *Gelidiella* species which shall be of the following two grades:
  - a) Grade I, and
  - b) Grade II.

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3. Requirements

Reference Community STEP Standards Information Coming and Aysor

- 3.1 Description The dried seaweed shall be in a whole or powder form. It shall be yellow-brown, grey-yellow to light-brown, or dark-brown to reddish-brown in colour. It shall be free from objectionable or rancid odour and insect infestation. The material shall have a characteristic smell of dried seaweeds.
- 3.2 Identification The material shall be identified by the description given in Appendix B.
- 3.3 The material shall also comply with the requirements given in Table 1.

SI No.	Characteristic	Requirements			Method of Test,	
		Gracilaria		Gelidiella		Ref to Appendix
		Grade I	Grade II	Grade	Grade II	
1)	Agar, percent by mass, <i>Min</i>	35	25	45	45	C-1
ii)	Gel strength*, g/cm², <i>Min</i>	125	70	280	200	C-2
iii)	Moisture, percent by mass, <i>Max</i>	18	20	18	20	C-3
įv)	Dirt, dust, mud and sand percent by mass, <i>Max</i>	5	<b>7</b>	5	7	C-4
v)	Other seaweeds, percent by mass, <i>Max</i>	3	5	3	5	<b>C</b> -5

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## IS: 12342 ( Part 1 ) - 1988

#### 4. Packing, Storage and Marking

- **4.1** Packing The material (powder or whole) shall be packed in plastic lined hessian, craft paper or plastic thread woven gunny bags. The bags shall be attached properly to protect the material from moisture and humidity.
- **4.2** Storage The material shall be stored in a cool dry place so as to avoid excessive exposure to heat and light.
- **4.3** Marking Each bag shall be suitably marked to give the following information:
  - a) Name of the seaweed,
  - b) Name and address of the supplier,
  - c) Net mass,
  - d) Month and year of collection, and
  - e) Any other requirement laid down under the Standards of Weights and Measures (Packaged Commodities) Rules, 1977.
  - 4.3.1 Standard marking Details available with the Bureau of Indian Standards.

#### 5. Sampling

- **5.1** Unless otherwise agreed to between the buyer and the seller, the sampling plan given in IS: 2500 (Part 2)-1965 'Sampling inspection tables: Part 2 Inspection by variables for percent defectives', may be followed. The inspection level and acceptable quality level (AQL) for various requirements shall be as given in **5.1.1** and **5.1.2**.
- 5.1.1 For agar and gel strength, a single sampling plan with inspection level IV and AQL 1.5 percent [see Table 1 and 4 of IS: 2500 (Part 2)-1965 'Sampling inspection tables: Part 2 Inspection by variables for percent defectives'] shall be used.
- 5.1.2 For remaining requirements, a composite sample made out of the bags examined according to 5.1.1 and found satisfactory shall be tested. All the test results on the composite sample shall meet the corresponding specification requirements.

#### 6. Tests

- 6.1 Tests shall be carried out by the methods specified in 3.2 and Table 1.
- **6.2** Quality of Reagents Unless specified otherwise, pure chemicals and distilled water [ see IS: 1070-1977 'Specification for water for general laboratory use ( second revision )' ] shall be employed in tests.

Note — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the results of analysis.

#### APPENDIX A

( Clause 0.2.1 )

#### DETERMINATION OF QUALITY INDEX AND PRICE FIXATION

A-1. Within the limits of recommended levels of seaweed constituents and impurities, a formula has been designed by which one can calculate the quality index (QI) of different seaweeds. The quality indices will be comparable for a particular type of phycocolloid produced by the same or different species.

Quality index = Content of phycocolloid (percent) x quality unit\*

Total impurities†, percent

A-2. Based on this index, different types of seaweeds can by compared and their market value fixed. For example, *Gracilaria* of Grade 1 quality having 35 percent agar, 125 g/cm² gel strength and 9 percent impurities (7 percent moisture, 1 percent dirt, dust, etc, and 1 percent unwanted seaweeds) will have a QI of 486 while second grade *Gracilaria* having 25 percent agar, 70 g/cm² gel strength and 18:5 percent impurities (14 percent moisture, 3 percent dirt, dust, etc and 1:5 percent unwanted seaweeds) will have a QI of 95. Both the quality indices are in the ratio of 5:1. Therefore, the market value of grade 1 *Gracilaria* should be five times that of Grade II quality.

<sup>\*</sup>Gel strength (g/cm²) for 1.5 percent agar at 27°C.

<sup>†</sup>Adhered foreign materials like dirt, dust mud and sand or adulteration by unwanted seaweeds, moisture, etc.

### APPENDIX B

( Clause 3.2 )

#### **IDENTIFICATION**

- **B-1.** Plants are with thalloid structure, having chlorophyll *a* and simple reproductive structures. Cells have cytoplasmic connections, cell divisions are rarely intercalary; carporspores are produced in carposporangia borne on gonimoblast filaments. Life history is usually with a free-living tetrasporophyte; sexual and sporophytic generations are generally isomorphic.
- **B-1.1** Gelidiella Gonimoblasts grow directly from a carpogonium fusion cell. Tetrasporangia are tetrahedral, tetrapartite, or irregularly divided. Rhizomes are absent. Branch tips have a single apical cell.
- **B-1.1.1** Plants are bushy, cartilagenous and terete; apex has a single apical cell organizing a multiaxial medulla of long thick walled cells and a cortical zone of a few layers of short cells outermost with chromatophores. Sporangia are usually in stichidial branchlets.
- **B-1.2** Gracilaria Gonimoblasts grow mostly from an auxiliary cell. Auxiliary cell is an intercalary vegetative cell. Texture is firmer, branches are essentially terete, branch tips erect and growth multiaxial. Tetrasporangia are tetrapartite; medulla is parenchymatous throughout. Tetrasporangia are scattered over the general branch system.
- **B-1.2.1** Plants are usually bushy from a small discoid base, terete or flattened, fleshy to cartilagenous dichotomously irregularly, or proliferously branched, and of pseudoparenchymatous structure and without filamentous cells in the mature vegetative thallus.

#### APPENDIX C

( Table 1 )

## METHODS OF TEST FOR AGAROPHYTES

#### C-1. Agar

C-1.1 Procedure — Weigh 100 g dry seaweed powder and place it in a 10-litre beaker and hydrate this for at least 3 hours with 7.5 litres of distilled water at pH 6.3. Cover the container, make a small vent hole, and autoclave for 3 hours at 121°C. Filter under pressure through glass-wool and a membrane filter (12  $\mu$ m). Keep the solution and the filter apparatus above 60°C. Pour the solution into shallow trays and allow it to gel at room temperature. Cut the gel into 2 cm strips and freeze. Thaw the gel at room temperature and filter off the free liquid with suction through an ordidary filter paper covered with hylon mesh. Wash the residue carefully with three 500-ml portions of distilled water and later on with 100 ml ethanol. Dry the agar overnight in an oven maintained at 60°C and weigh. Store in a dry place. Triplicate samples shall be extracted in this way. The quantity of extractable agar, in these samples, shall not vary by more than 1 percent. Take the mean of triplicate quantity.

#### C-1.2 Calculation

Agar, percent by mass = 
$$\frac{M_1}{M_2} \times 100$$

where

 $M_1 = \text{mass}$ , in g, of the agar; and

 $M_2$  = mass, in g, of the material taken for the test.

## C-2. Strength of Agar Gel

## C-2.1 Apparatus

C-2.1.1 Gel strength tester

## IS: 12342 ( Part 1 ) - 1988

#### C-2.2 Procedure

- C-2.2.1 Take a 500-ml beaker and determine its weight. Weigh 3 g agar in this beaker. Add the required amount of distilled water to this beaker so that the combined weight of agar and water is 200 g. Boil the water till agar dissolves completely. Again weigh and adjust the combined weight of agar and water to 200 g with hot distilled water. Pour the agar solution into a 300-ml beaker of 5 cm diameter. Allow it to gel at 27°C (room temperature) for 15 hours before measuring gel strength. Prepare three sets in this way.
- C-2.2.2 Fix the gel strength tester in a horizontal position. Move the handle to right side for raising the weight adding device. Place the beaker, containing jelly, in such a way, that the lower surface of the weight adding device (having 1 cm² base) should be in the centre of the beaker. Place an appropriate weight on the weight adding stand. Move the handle in the reverse direction and lower it gently until the end of the weight-adding device comes in contact with the jelly. Start the stop-watch as soon as the handle supporting the weight-adding device is separated from it and the weight is given to the jelly completely. Record the time from the beginning of the cracking of jelly till the entry of the end of the weight-adding device into jelly. If it penetrates within 20 seconds, change the measuring position and decrease the number of counter weights and repeat the measurement once again. If it does not penetrate after a lapse of 20 seconds, add another suitable weight and make the measurement as described earlier; repeat measurement till the maximum weight is obtained which takes 20 seconds for penetration. After measurement, add the weight of weight-adding device to the counter weight (weights added during measurement). This value is the strength of jelly (in g/cm²).
- C-2.2.3 Take triplicate measurements. The values should not vary by more than 1 percent. Calculate the mean of the triplicate measurements.

#### C-3. Moisture

C-3.1 Procedure — Take about 10 g of air-dried seaweed sample into a weighed porcelain dish. Weigh and place in an oven maintained at  $105 \pm 2^{\circ}$ C. Dry it at this temperature for 6 hours. Cool in a desiccator and weigh. Carry out triplicate determinations for the samples collected at random from one lot. The results of triplicate readings shall not vary by more than 1 percent. Determine the mean of triplicate results and calculate the moisture in percent.

## C-3.2 Calculation

Moisture, percent by mass 
$$=\frac{(M_2-M_1)}{M_2}\times 100$$

where

 $M_1 = \text{mass}$ , in g, of the material after drying for 6 hours; and

 $M_2$  = mass, in g, of the material taken for the test.

#### C-4. Dirt, Dust, Mud and Sand

- C-4.1 Procedure Weigh 250 g air-dried seaweed and soak it for 30 minutes in one litre of tap water (salt content not more than 300 mg/kg) in a beaker. Gently rub and agitate the seaweeds in the water to dislodge the adhered materials. Hand pick the seaweed from the water. Then filter the water through double fold muslin cloth. Remove the insoluble matter from the cloth and keep it separately. Remove the fragements of seaweeds, if present. Keep the filterate in another container. Again wash the same seaweeds with another one litre quantity of tap water. Separate the insolubles and filterate as described earlier. Combine the two insoluble matters into one. Similarly combine the two filterates into one.
- C-4.1.1 Wash the insoluble matter with distilled water. Transfer the washed and dewatered insoluble materials into a weighed crucible and dry it at 105  $\pm$  2°C in an oven and weigh.
- C-4.1.2 Find the total volume of combined filterate. Mix thoroughly and pipette 100 ml of the filterate with the help of a volumetric pipette. Transfer this aliquot to a previously weighed porcelain basin. Dry it completely on a water bath. Then transfer it to a muffle furnance maintained at  $400^{\circ}\pm5^{\circ}$ C. Maintain at this temperature for 6 hours. Remove the basin from the furnace when the temperature is about 150°C. Cool it in a desiccator at room temperature and weigh. Calculate the quantity of inorganic materials for total combined filterate. Calculate the percentage of dirt, dust, mud and sand.
- C-4.1.3 Make triplicate analysis for each lot. The samples should be collected at random. The results of the triplicate analysis should not vary by more than 5 percent. Calculate the mean of the triplicate analysis.

#### C-4.2 Calculation

Dirt, dust, mud and sand, Percent by mass =  $\frac{(M_1 + M_2)}{M} \times 100$ 

where

M = mass, in g, of the material taken for test;

 $M_1$  = mass in g, of the insoluble material (C-4.1.1); and

 $M_2 = \text{mass}$ , in g, of the inorganic material (C-4.1.2).

**C-5. Other Seaweeds** — Contaminating weeds are generally green seaweeds like species of *Ulva Rhizoclonium*, etc. Sometimes red seaweeds like *Hypnea* are also adulterants. This alga can easily be identified by its short cylindrical filaments with spiny appendages on the main axis and sometimes the presence of hook shaped tendrils on the apex.

C-5.1 Procedure — Take 1 kg of air-dried seaweed sample. Isolate the contaminants manually and determine their total mass. Calculate the percentage contaminants. Make triplicate determinations for the samples collected at random. The results of the triplicate readings should not vary by more than one percent. Calculate the mean of the triplicate readings.

#### EXPLANATORY NOTE

Seaweeds are commercially utilized for the extraction of chemicals like alginic acid, sodium alginate (textile and pharmaceutical grades) and agar (food and microbiological grade). These products have widespread usage in food, pharmaceutical textile, confectionery, cosmetic and other industries. The raw seaweeds also have a good potential for export.

The availability of these seaweeds is less than the demand and, therefore, these are adulterated, the common adulterants being moisture, dirt, mud, sand, etc, and low quality seaweeds of the same or different species. This standard has, therefore, been formulated to ensure availability of seaweeds of of the desired quality.

Agar is used in microbial cultures, food, medicine drugs and pharmaceuticals, and in canning. It is also used as a stablizer, thickener and gelling agent and for sizing of fabrics, etc. Its use as an ingredient in waterproof paper, cloth and glue, as a clarifying agent in beer and wines and in the preparation of special diabetic foods is also very common. The following Indian Standards have been published on agar for different purposes:

IS: 5707-1970 Agar, food grade

IS: 6850-1973 Agar, microbiological grade

In the preparation of this standard, considerable assistance has been obtained from Central Salt and Marine Chemicals Research Institute, Bhavnagar.

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test, shall be rounded off in accordance with IS: 2-1960 'Rules for rounding off numerical values (revised)'. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.